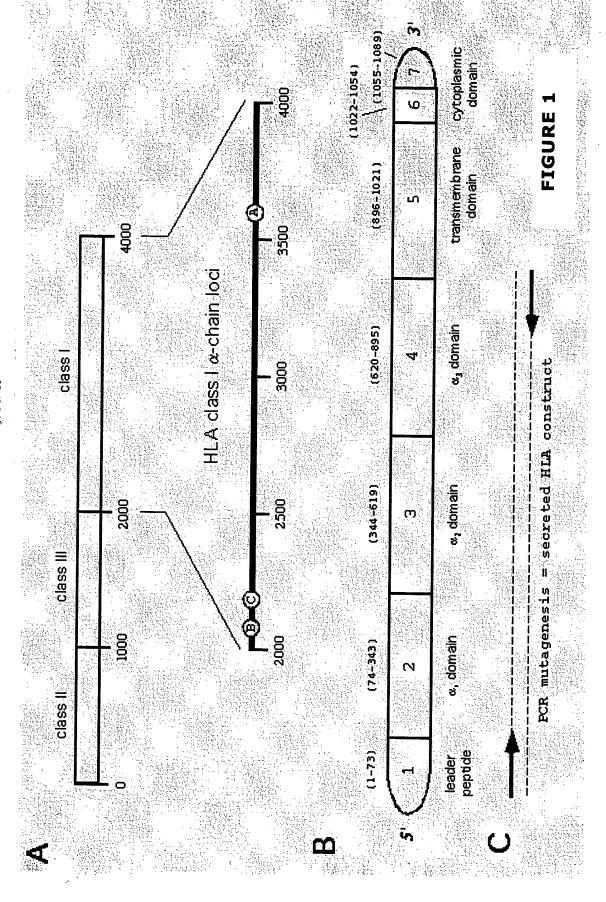
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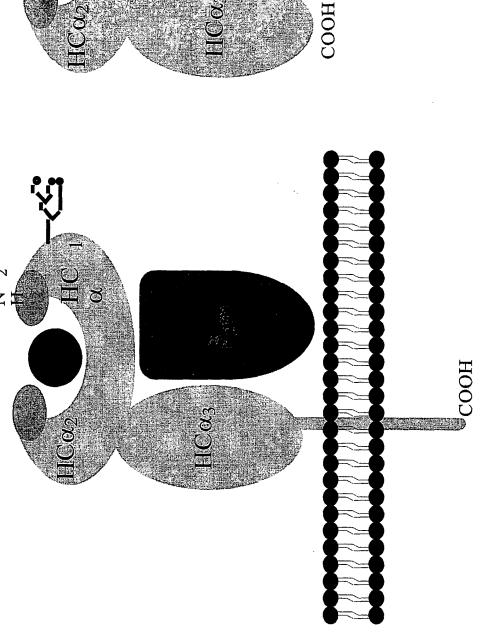
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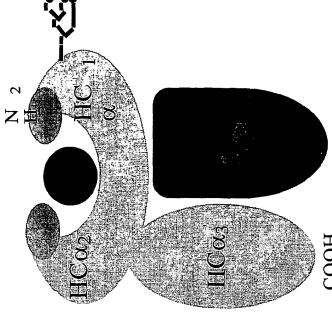
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Native, transmembrane HLA molecule



Recombinant, truncated sHLA molecule

FIGURE 2

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SIDE VIEW **TOP VIEW**

FIGURE 3

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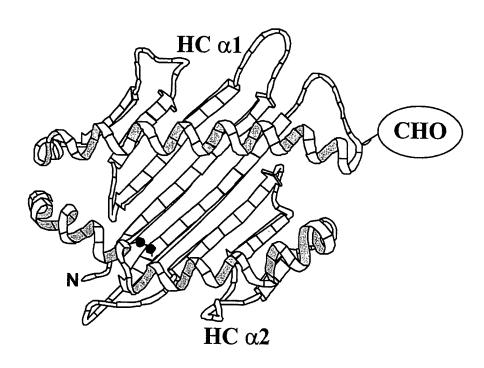


FIGURE 4

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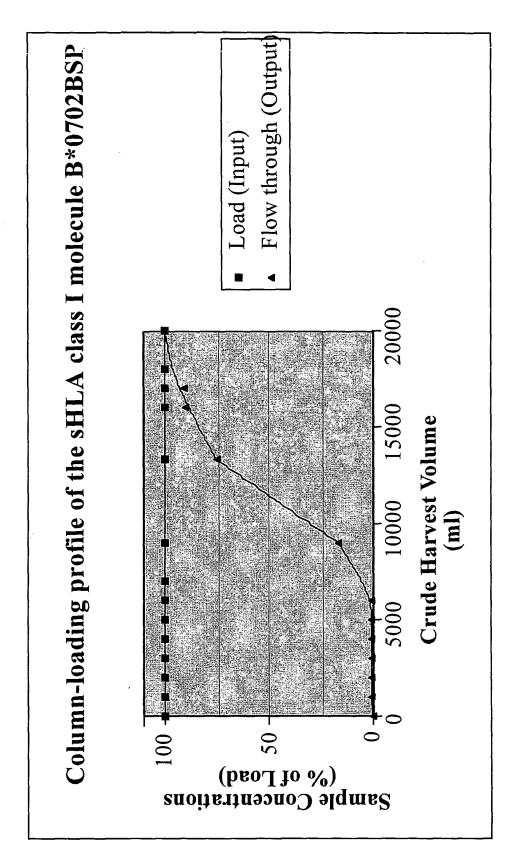
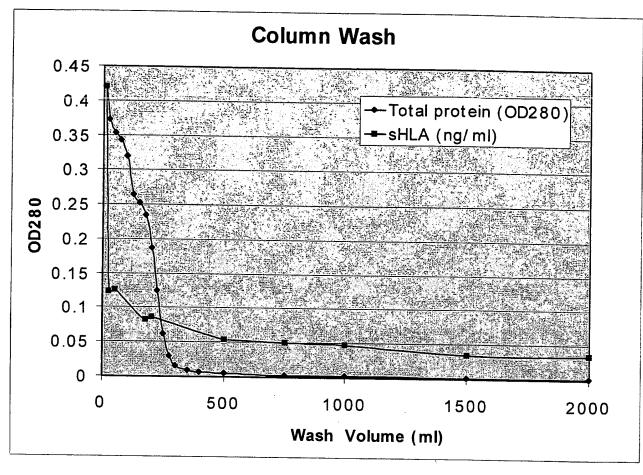


FIGURE 5



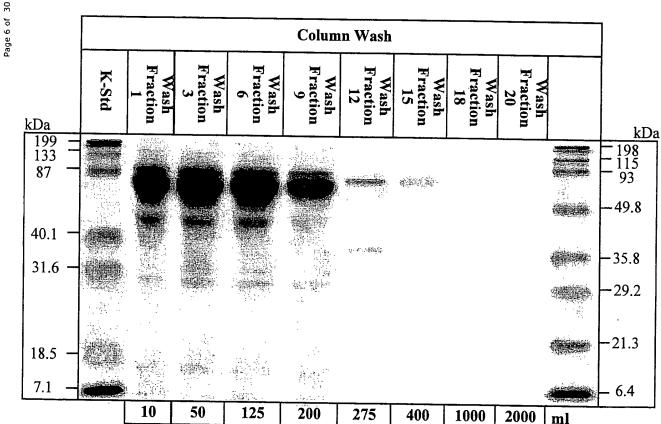


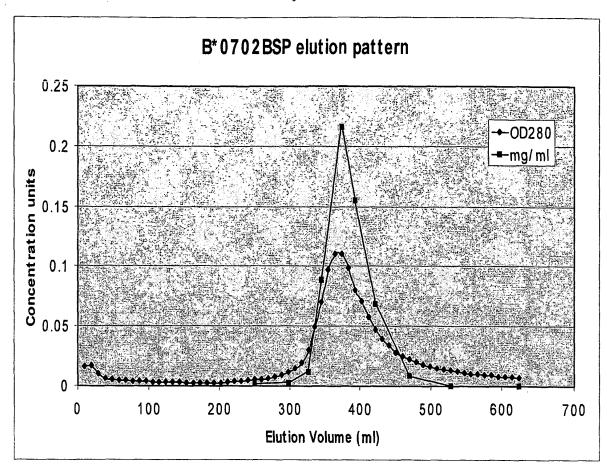
FIGURE 6

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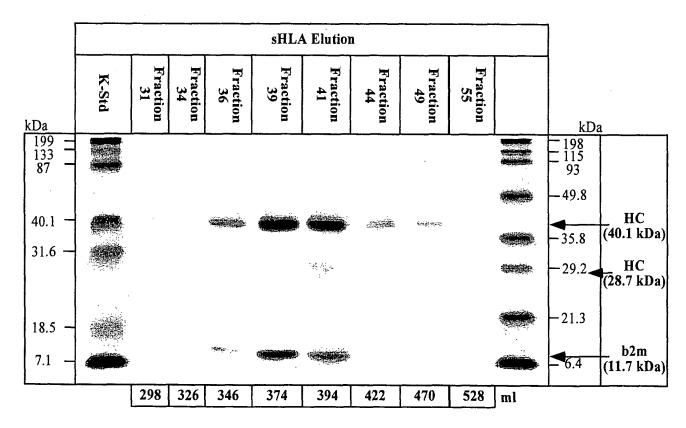


FIGURE 7

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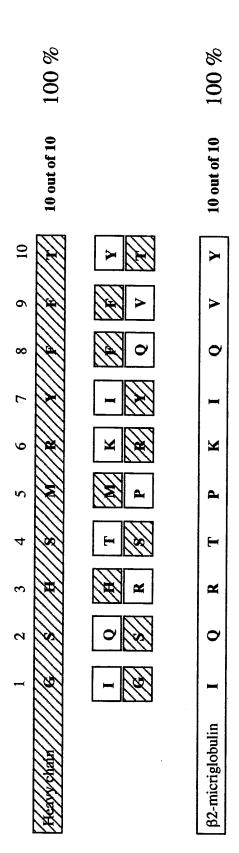


FIGURE 8

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% Difference 22.6 19.4 4.3 2.5 8.6 9.5 6.4 5.4 ∞ 2.8 9.0 Observed residues 47.8 34.9 17.9 26.2 11.3 13.2 28.5 30.5 24.6 21.1 22.3 18.6 20.0 4.7 ŧ 333 aa Calculated residues **7**6 23 50 12 76 333 9 13 1013 Aspartic acid + Asparagine Glutamic acid + Glutamine **Phenylalanine** Methionine **Iryptophan** Isoleucine **Threonine** Histidine Arginine Cysteine **Tyrosine** Glycine Alanine Leucine Proline Lysine Valine Serine Total Glu+Glu Asp+Asn Cys Ala Phe Arg GIV His Lys Len Met Pro He Thr Trp Ser Val Tyr H Σ 2 S 13 5 13 14 16 4 9 ∞ ∞ 6 6

FIGURE 9

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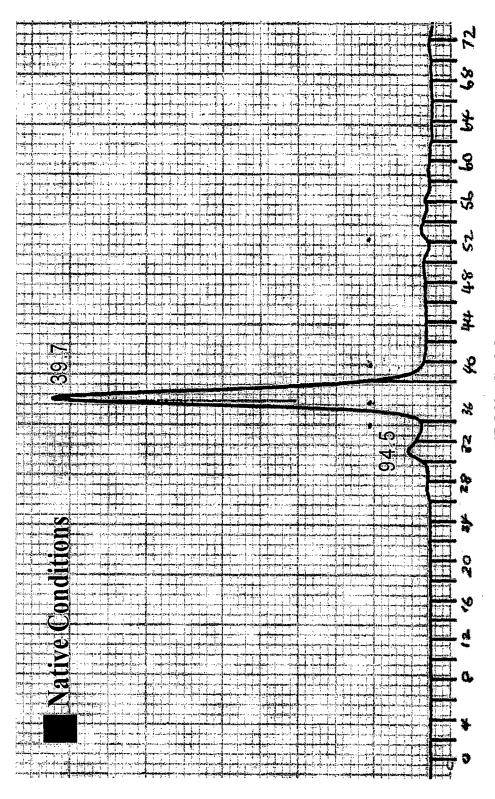


FIGURE 10

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cts 58 63 6.95 ation 2 0 82m 15.1 Complex 35.6 တ တ aggregate. 109 विष्टेहार्ट्डवर्ग | 94 ह 83 3

FIGURE 11

85

----- o

b2-microglobul

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Coomassie-stained SDS-PAGE loaded with 2µg of protein

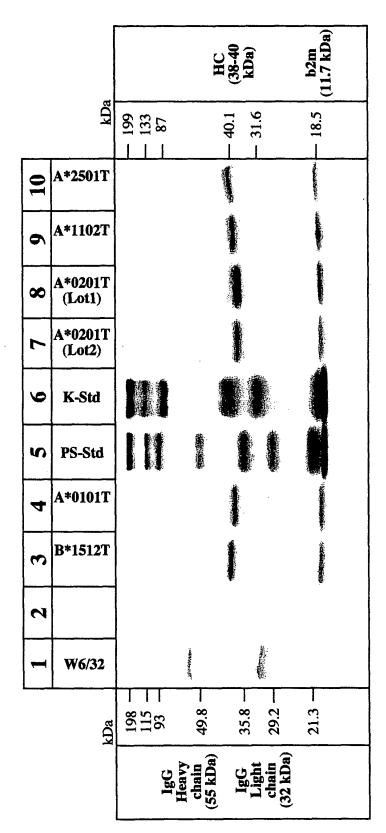


FIGURE 12

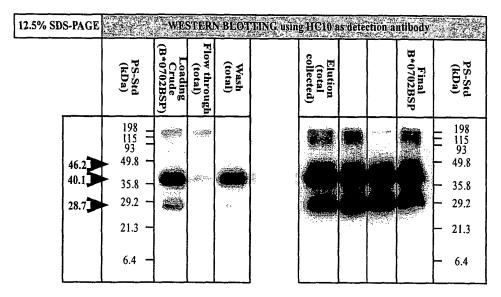
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SDS-PAGE using Coomassie blue as staining method 12.5% SDS-PAGE Loading Crude (B*0702BSP) Flow through (total) Concentrate (B) Concentrate (A) Final B*0702BSP Elution (total collected) PS-Std PS-Std (kDa) Wash (total) PS-Std (kDa) K-Std 198 115 93 49.8 198 115 93 Growth **953** medium, proteins. 49.8 35.8 35.8 29.2 29.2 28.7 21.3 21.3 **E** 11.7 6.4 6.4



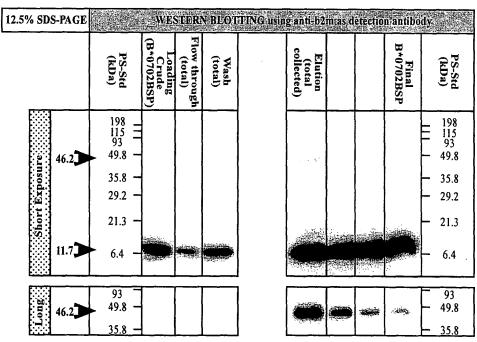


FIGURE 13

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B*1510T/pcDNA3 B*1508T/pcDNA3 B*1503T/pcDNA3B*1501T/pcDNA3:2 B*1501T/pcDNA3:1 B*I201T/PSR B*1201/PSR B*1501T/pcDNA3/G418ex B*1501T/pcDNA3/G418 B*1201T/PSR/G418

calreticulin 60 kDa

FIGURE 14

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B*1501 Peptide-binding specificities

sHLA*1501

HLA*1501

Amino acid position of peptides										Amino acid position of peptides								
1	2	3	4	5	6	7	8	9	`	1	2	3	4	5	6	7	8	9
	Q	K	D	I				Y				K	D	1				Y
	L	P	₽	V	•••••			T		*****		P	P E		5565656 	1600000	994999	F
	P	R	G									R						
	V	F										F	-					
			•	1								N Y						

FIGURE 15

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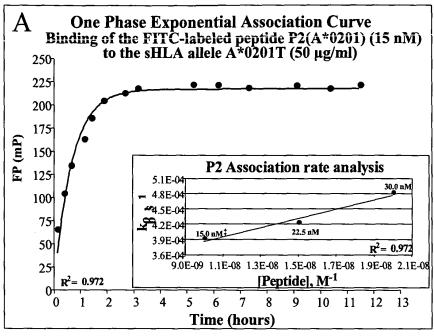
fluorescent ligand High FP "Bound" fluorescent ligand Low FP "Free" Parallel and Perpendicular Fluorescent Molecule Incident Polarized light

FIGURE 16

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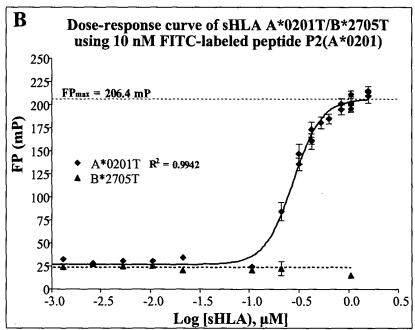
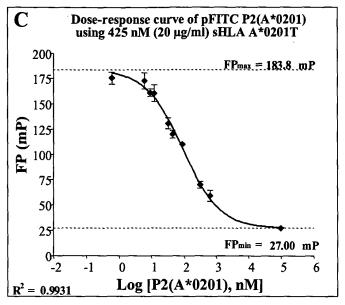


FIGURE 17

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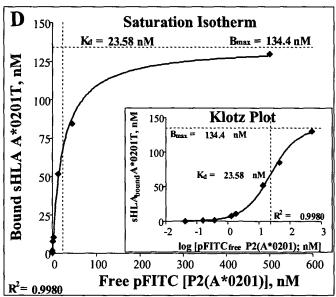


FIGURE 17 CONT'D

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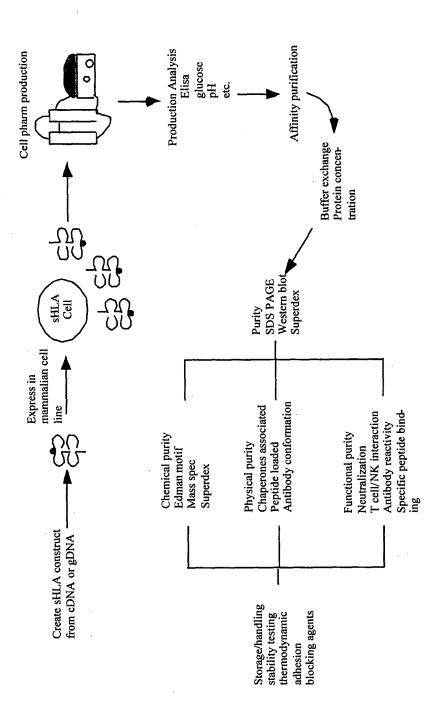


FIGURE 18

STEP 1: Choice of appropriate support

STEP 2: Coating with pan HLA specific antibodies





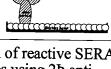
STEP 3: Blocking procedure to reduce non-specific protein binding

STEP 4: Capturing of single specificity sHLA molecules at different epitopes



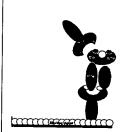




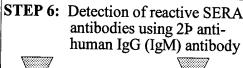


STEP 5: Positive (or negative) SERA binding to

presented sHLA alleles











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Z anti-β2m **FIGURE 20** ■ W6/32 DHC10 [m/g4 [.0 using a gradient of sHLA concentrations directly coated to an ELISA plate [m/g4 2.0 Activity confirmation of B*1512T [m/g4 4.0 [m/g4 8.0 lm/g4 0.1 1m/g4 2.£ Concentrations [m/g4 4.0 12.8 µg/ml Im/gy 02 100 µg/m] 0.5 Absorbance (1 1.5 2.5

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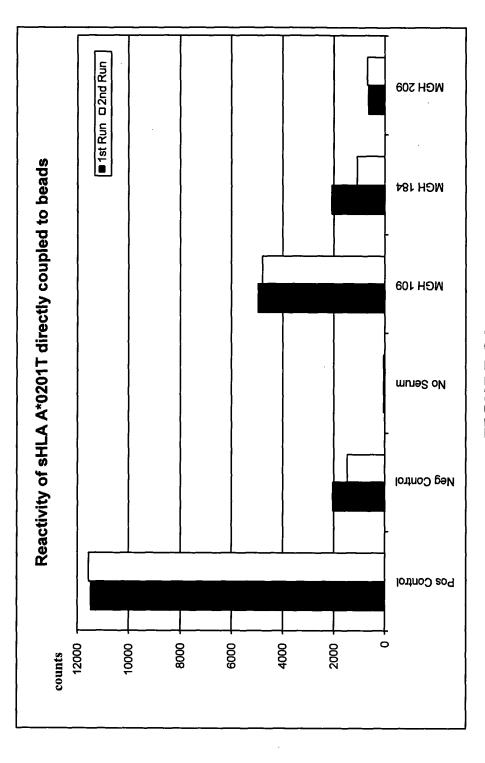


FIGURE 21

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□ anti-b2m ■ W6/32 B*2705T B*0702T (1:500) IgG2a Antibody-Reactivity A*7401T (BIH0037) against a selection of Pure Protein's sHLA molecules tested using two different capturing methods A*0101T B*1501T Selected Test-Alleles A*2501T B*3505T A2/A28 A*2402T 3% BSA ŝ One Lambda A*6901T A*6801T A*0201T ND = Not determined4 3.5 2.5 **OD**†80

FIGURE 22

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□ anti-b2m ■ W6/32 B*5001T B12 (1:500) IgG2b Antibody-Reactivity (BIH0066) against a selection of Pure Protein's sHLA molecules tested using two different capturing methods 3% BSA Selected Test-Alleles B*4501T One Lambda B*4402T 4 m 3.5 2.5 1.5 0.5 **OD**480

FIGURE 23

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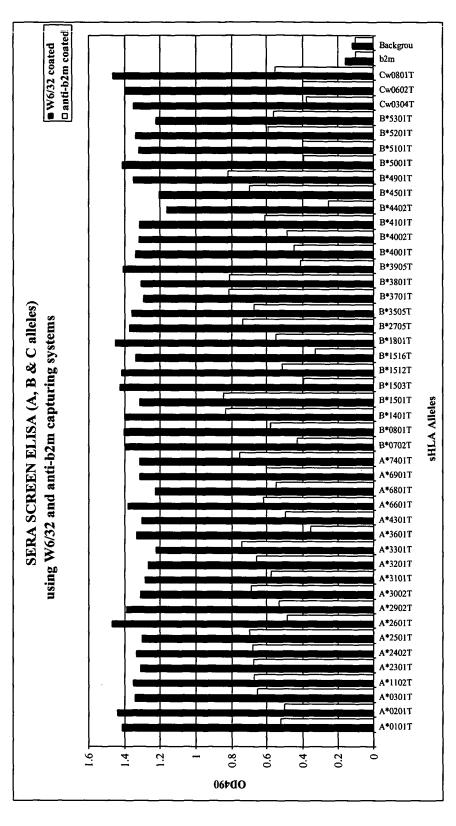


FIGURE 24

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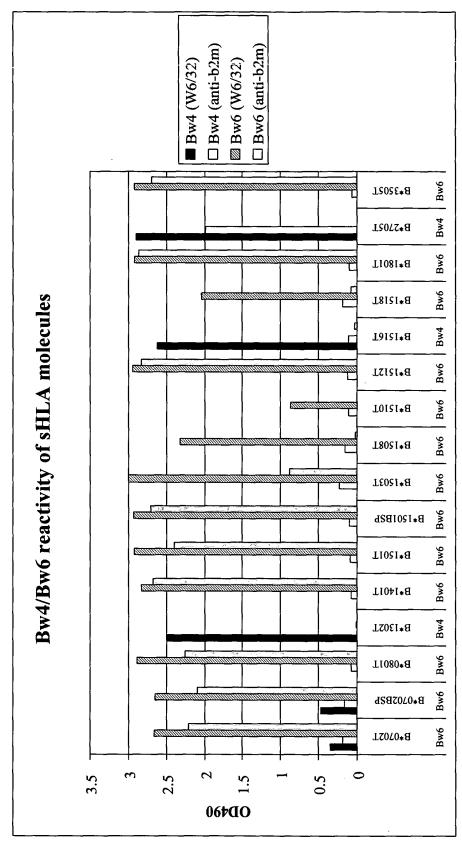


FIGURE 25

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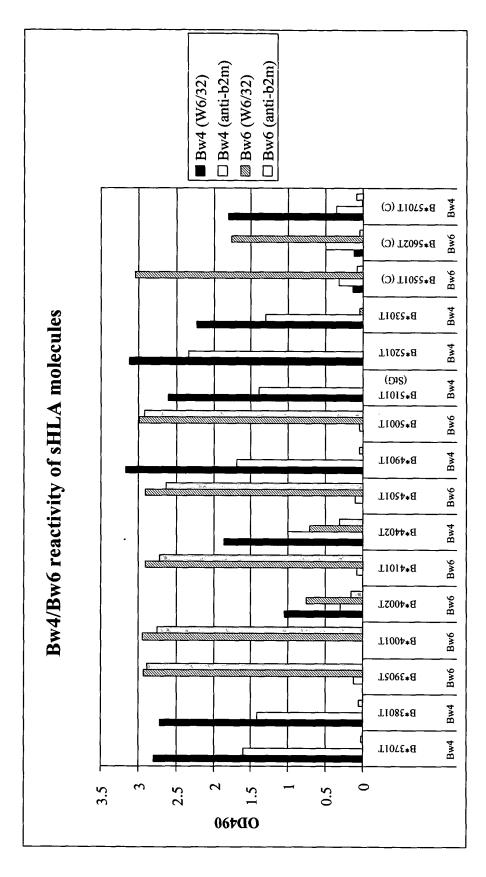


FIGURE 26

□ TP25.99 **FIGURE 27** ☑ 2Þ only ■ W6/32 ■ HC10 ELISA procedure to test capturing efficiency lm/gn 2.21 lm/gn 22 Page 28 of 30 so ng/ml Serial No. Not Yet Assigned Titles ANTI-HLA ASSAY AND METHODS Applicant: William Hildebrand Atty: Kathryn L. Hester, Ph.D. Serial No. Not Yet Assigned sHLA A*0201T concentrations 100 ng/m] 1m/gn 002 1m/gn 004 1m/gn 008 0.2 9.0 0.4 8.0 1.2 1.4 Absorption

Dkt. No. Tel: Filed:

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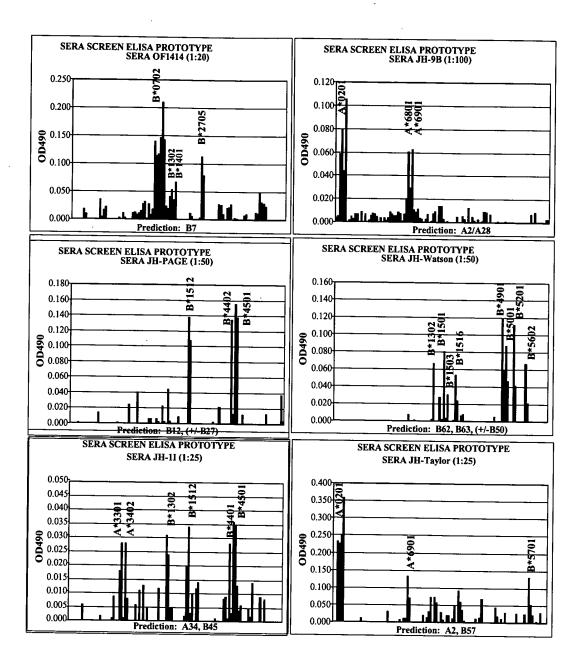


FIGURE 28

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